Two new species of Bryonectria (Hypocreales, Ascomycetes) on bryophytes

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Abstract:
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Two bryophilous species of the genus *Bryonectria* (Hypocreales, Ascomycetes) are described as new and illustrated. *B. callicarpa* from Europe characterized by orange-coloured perithecia infects *Frullania dilatata* (Hepaticae). *B. phyllogena* on *Polytrichum juniperinum* (Musci) from Southern America and Europe has brownish perithecia covered by densely interwoven hyphae. In both species inconspicuous phialides representing the anamorphic state are recorded.

Zusammenfassung:
Zwei bryophile Arten der Gattung *Bryonectria* (Hypocreales, Ascomycetes) werden neu beschrieben und abgebildet. *B. callicarpa* aus Europa zeichnet sich durch orangefarbene Perithecien aus und infiziert *Frullania dilatata* (Hepaticae). *B. phyllogena* auf *Polytrichum juniperinum* (Musci) aus Südamerika und Europa hat bräunliche Perithecien, die von einem dichten Hyphenmantel bedeckt sind. Bei beiden Arten werden unauffällige Phialiden als anamorphe Stadien nachgewiesen.

In the course of further studies on bryophilous ascomycetes two additional members of the genus *Bryonectria* Döbbeler (Bionectriaceae, Hypocreales) were detected by screening potential hosts. They are presented as new species which considerably extend our concept of the genus.

Bryonectria callicarpa Döbbeler, **spec. nov.** (Fig. 1, 2)*

Holotype: Spain, Prov. Gerona, Catalonian Pyrenees, surroundings of the main road N 260 about 20 km west of Figueres in the direction of Olot, on the bark of trees, on *Frullania dilatata*, 20.8.1999, *Döbbeler 7094* (Holotype M, isotype BCC).

Etymology: *callicarpus* from Greek *kallikarpos* = with beautiful fruits, refers to the fruit-bodies.

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* All measurements (except ascomata size) and illustrations were made from slide mounts in lactophenol cotton blue.

Ascomata 120–150 × 85–130 µm, conica vel subpyriformia, aurea ad luteola (in statu vivo), sine setis. Paries ascomatum lateraliter 12–20 µm crassus, e cellulis crassitunicatis irregularibus formatus. Paraphyses apicales praesentes. Hyphae inter ascos deficientes. Asci 52–70 × 8–10 µm, cylindrici, in apice sine anulo, primo 8-spori, maturitate 4-spori. Ascosporae 12–14 × 4–5 µm, ellipsoideae, 2-cellulares, incoloratae sed in quaque cellula guttula luteola praeditae, episporio leniter cyanophilo. Hyphae 2,5–5 µm crassae, incoloratae, crassitunicatae, supra cellulas hospitis crescentes, appressoriis lateralibus, sessilibus, rotundatis, 5–8 µm diametientibus ornatae. Status anamorphus: Phiales 5–8 × 2–3,5 µm, ampulliformes, partem concavam interioremque texturarum hypharum 20–60 µm diametientium vestitae. Phialosporae perminutae.

Habitat parasitice in plantas vivas hepaticae *Frullania dilatata*. Ascomata saepe laxe aggregata, omnino in parte ventrali hospitis sita.

Ascomata perithecial, (105–)120–150(–175) × 85–130(–150) µm, conical to nearly pyriform, orange red to yellowish, externally paler, dead herbarium material bleaching and finally colourless, without setae, surface uneven by shortly protruding groups of cells; no colour change in KOH; ascomata not cupulate when dry but laterally collapsing. Ascomatal wall in surface view normally without recognizable cells; in section wall laterally 12–20 µm thick, composed of irregular, thick-walled hyphal cells with small, reduced lumina; outer cells almost colourless, inner ones with yellowish guttules. Apical paraphyses arising from the inner wall of the middle and upper part of the ascomata, filiform, up to 60 µm long and 2–3 µm wide, only basally ramified, further up as periphyses lining the ostiolar canal; filaments rich in orange-yellow guttules. Interascal hyphae absent. Asci 52–70 × 8–10 µm, cylindrical, straight or curved, the apex rounded and without a ring, foot short and thick; mature asci 4-spored. Ascospores (11–)12–14(–15) × 4–5 µm, ellipsoidal, 2-celled, not constricted at the septum, colourless, but with a large yellow lipid body in each cell and often a smaller one additionally, epispore slightly cyanophilic; 8 ascospore-initials are visible in young asci of which only 4 reach maturity whereas the other 4 spores remain smaller, sometimes even insert a septum but do not develop further and finally degenerate. The distribution of the favoured spores within the asci is quite irregular. Hyphae 2.5–5 µm wide, colourless, thick-walled, with appressoria, growing superficially over the host cells, prefering the anticlinal cell walls, branched and anastomosing; in heavily infected leaves hyphae forming plates which may completely cover individual host cells. Appressoria 5–8(–10) µm diam, laterally arising from the hyphae, sessile, more or less circular in outline, preferably formed over the junctions of the anticlinal cell walls. Anamorphic state: subhemispherical aggregations of hyphal cells leaving a small hollow space between themselves and the leaf surface, seen from above roundish or slightly irregular, 20–60 µm diam, about 20 µm high, inner side of the dome-cells covered with 5–8 µm long and 2–3.5 µm wide, bottle-shaped phialides, phialospores wedge- or rod-like, up to 4 × 0.5–1 µm; ostiolar opening absent; the phialides producing structures are difficult to distinguish from perithecial primordia.

Host: *Frullania dilatata* (L.) Dumort.

Known distribution: Ireland, Germany, Spain

Localization and biology:

The fruit-bodies are restricted to the ventral side of the infected plants, that is the stems, underleaves and water sacs as well as the lateral leaves prefering the peripherical regions. They

are often loosely aggregated, laterally attached and irregularly oriented. The host shows a slightly reduced vitality and algae may be present. Nevertheless, a negative effect by the parasite seems to be unlikely.

In the type material several apothecia of *Pithya frullaniae* Chalaud could be observed growing sporadically on dead parts of *Frullania dilatata* (see CHALAUD 1942). Neither *Bryonectria callicarpa* nor *P. frullaniae* have been proven on associated plants of *Radula complanata* (L.) Dumort.

Remarks:

Even plants with many coloured ascomata are not recognizable as infected in dorsal view because the perithecia exclusively develop on the ventral side. It is indispensable to screen the *Frullania*-mats from the underside or to turn over individual plants or shoots.

Two remarkable features of this species are the irregular orientation of the fruit-bodies pointing in all directions, combined with the hidden and therefore relatively protected position within the host-mats. Directed ascospore liberation into the open air apparently does not occur. The development of only four ascospores seems to indicate a reduced fertility though fourspored asci are known in other *Bryonectria*-species as well. Whether the loosely attached perithecia represent distribution units could not be proven. It is also unclear how the phialoconidia produced within the dome-like cavities of hyphal cells are liberated.

Bryonectria callicarpa is a distinctive species recognizable, apart form microscopic details, by its orange red to yellowish perithecia (at least in the vital state) borne on the ventral side of *Frullania dilatata*. All other species of *Bryonectria* so far named have uncoloured or hyaline perithecia. However, coloured perithecia have been observed several times in undescribed species of *Bryonectria*. Whether the coloration disappears completely in herbarium material is not known. The reddish *Nectria egens* Corner on the epiphyllous hepatic *Leptolejeunea vitrea* (Nees) Schiffn. from Malaya seems to be congeneric, judging from the detailed description and illustrations by CORNER (1935).

Further specimens examined:

Ireland. Co Galway (H 16), Lough Corrib, Archipelago off N shore of Dooras Peninsula „Derry Rock North", 53°30'N 9°22'W, 0–3 m, 1.5.1998, *Hertel 39525 b* (M).

Germany. Bavaria: Allgäu, Kreis Sonthofen, Hinterstein, 1000 m, 28.7.1949, *Grützmann* (M).

Bryonectria phyllogena Döbbeler, **spec. nov.** (Fig. 3, 4)

Holotype: Chile, VIII. Región del BíoBío, Prov. de Ñuble, Nevados de Chillán, Refugio Aserradero, ca. 1300 m, on *Polytrichum juniperinum*, 5.4.1987, *Grau* (M).

Etymology: *phyllogena* from Greek *phyllon* = lamella and *genos* = origin, referring to the fruit-bodies arising from hyphae within the lamellar interspaces of the host leaves.

Ascomata 190–250 × 150–200 µm, conica vel pyriformia, dilute brunnea ad fuliginea, sine setis sed hyphis adhaerentibus. Ostiolum zona pallida, 40–100 µm diametienti circumdatum. Hyphae adhaerentes 15–50 × 3–4 µm, luminibus reductis, irregulares, stratum densum formantes. Paries ascomatum e cellulis nonnihil irregularibus crassitunicatis compositus, lateraliter usque ad 60 µm crassus, stratum externum hypharum 10–30 µm crassum. Canalis ostioli periphysibus vestitus. Hyphae inter ascos deficientes. Asci 75–95 × 10–13 µm, subcylindrici, sine structura apicali, plerumque 8-spori. Ascosporae 10,5–14 × 6–7,5 µm, ellipsoideae, 2-cellu-

lares, incoloratae, episporio subcyanophilo. Hyphae 2,5–5 µm crassae, incoloratae, crassitunicatae, in spatiis inter lamellas supra cellulas hospitis repentes, appressoriis lateralibus, sessilibus, ovoideis vel late ellipticis, 8–12 × 6–8 µm magnis, praeditae; hyphae saepe densae et pelliculas parvas formantes. Status anamorphus: Phiales 9–16 × 3–6 µm, ampulliformes ad subcylindricae.

Habitat parasitice in latere adaxiali foliorum musci *Polytrichum juniperinum*. Ascomata saepe aggregata et lateraliter coalescentia.

Ascomata perithecial (175–)190–250(–300) × 150–200(–220) µm, conical or pyriform, light to dark brown, rarely nearly colourless, overmature blackish-brown; ostiole surrounded by a light region, 40–100 µm diam, basal parts of ascomata often also paler; without projecting setae, but surface uneven and tomentose with adjacent hair-like hyphae; no colour change in KOH. Adjacent hyphae about 15–50 × 3–4(–5) µm, straight or flexuous, thick-walled with reduced lumina, generally light brown, irregularly arranged, forming a dense layer obscuring the ascomatal wall cells; apical hyphae elongated in a longitudinal direction, colourless, basally merging into the mycelial hyphae. Ascomatal wall in section laterally up to 60 µm thick, outer hyphal layer 10–30 µm thick; wall cells somewhat irregular with branches and anastomoses, but mainly tangentially elongated, externally with strongly thickened walls, lumina 2–10(–15) µm long. Ostiolar canal lined by plasmatic hyphae up to 35 µm long and 2–3 µm wide. Interascal hyphae absent, but empty collapsed asci may be mistaken for paraphyses. Asci 75–95 (–105) × (9–)10–13 µm, subcylindrical, apical structures not discernible, with an attenuated foot; mature asci with (5, 6, 7) 8 spores, initially always 8-spored. Ascospores (9–)10.5–14 (–16) × 6–7.5(–8) µm, ovoid to broadly ellipsoidal, 2-celled, septum delicate, not constricted at the septum, colourless, episporium presumably finely rough and somewhat cyanophilic. Hyphae (2–)2.5–5(–6) µm wide, colourless, thick-walled, with appressoria, growing superficially and irregularly over the host cells or prefering the anticlinal cell walls, branched and anastomosing, often forming strands; extended hyphal plates of appressorium-like cells are formed in heavily infected leaves; mycelium restricted to the lamellar region of the leaf, especially within the interlamellar spaces. Appressoria 8–12(–13) × 6–8 µm, laterally arising from the hyphae, sessile, seen from above roundish or broadly elliptical, often slightly sinuate. Anamorphic state: phialidic cells born laterally on the hyphae, 9–16 × 3–6 µm, bottle-shaped to subcylindrical, often two or few phialides aggregated; sometimes difficult to distinguish from hyphae torn during preparation; phialospores not seen.

Host: *Polytrichum juniperinum* Hedw.

Known distribution: Poland, Chile

Localization and biology:

Ascomata of *Bryonectria phyllogena* are formed on leaves in the middle or lower parts of the host sometimes showing signs of beginning decomposition. They occupy exclusively the adaxial leaf side and arise from the interlamellar spaces or sit upon the lamellae. Ascomata are often aggregated and tend to coalesce laterally. Those situated on the lowermost part of the lamina are laterally attached and point upwards to the leaf apices, whereas the other ones are irregularly oriented when formed on the free-lying lamellae. When developing below the inflexed leaf margins they face towards the slit between them, or they bend the margins upwards in order to discharge the ascospores without hindrance into the open air. Heavily infected leaves may yield up to 45 ascomata including initials. Even in these cases no adverse

effect on the host can be observed. The species is habitually difficult to distinguish from the southern hemisphere *Potridiscus polymorphus* Döbbeler & Triebel (Leotiales) which occurs on the same host species (Döbbeler & Triebel, in press).

Remarks:

Bryonectria phyllogena is not closely related to any other species of the genus. It has by far the largest fruit-bodies which additionally deviate by its brown colour and hyphal covering. Two further species are known on Polytrichaceae. *B. biseptata* Döbbeler on *Dawsonia* R.Br. from New Guinea and on *Polytrichum* Hedw. from Nepal is distinguished by asci with four biseptate spores (Döbbeler 1978, 1981). *B. cuneifera* Döbbeler on *Polytrichastrum formosum* (Hedw.) G.L.Sm. from Europe and *B. cuneifera* var. *jamaicensis* Döbbeler on *Polytrichadelphus flexuosus* (Müll.Hal.) Mitt. from Jamaica has hyaline and completely smooth though verruculose perithecia up to 130 µm diam (Döbbeler 1978).

At least a superficial resemblance exists with *Ticonectria perianthii* Döbbeler, a monospecific hypocrealean genus attacking the perianths of the epiphyllous *Radula flaccida* Lindenb. & Gottsche (Döbbeler 1998). *Ticonectria* Döbbeler is characterized by a different excipulum structure, partly intercellularly growing hyphae, missing appressoria and a different anamorphic state.

Further specimens examined:

Poland. Pomerania Occidentalis: distr. Chojnice, in pineto sicco ad lacum „Trzemeszno" prope pagum Mecikal, 3.10.1963, *Lisowski* (M, Bryoth. pol. 1725, sub *Polytrichum juniperinum*).

Chile. In locis soli expositis, prope Corral, puerto de Valdivia, *Krause* (M). – Patagonia occ., in valle fl. Aisén, 18.2.1897, *Dusén 588* (M).

The genus *Bryonectria* comprises seven species which grow on systematically diverse bryophytes in various ecological habitats (Döbbeler 1998, Döbbeler & Hertel 1984). Although the host range of the genus is characterized by an assemblage of unrelated tropical and non-tropical mosses and hepatics, individual species are restricted to single host species or a group of related hosts. Accidental records from geographically remote areas of the world and other observations indicate that many more members of the genus exist. Presumably, *Bryonectria* will prove to be one of the most species-rich relationships within the Hypocreales and ascomycetes on bryophytes.

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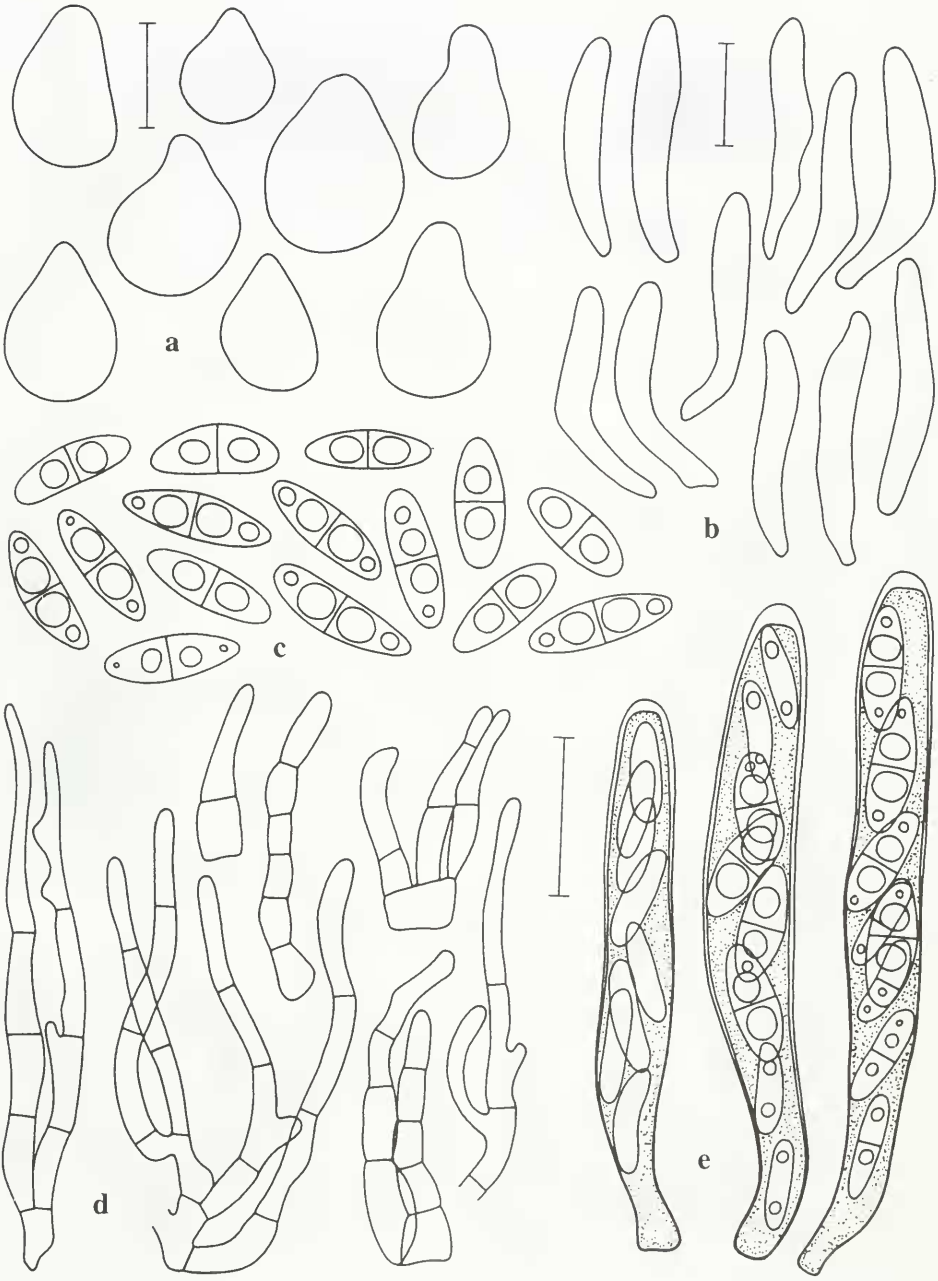

Fig. 1: *Bryonectria callicarpa* (M, holotype). a: Ascomata in outline; b: Asci in outline; c: Ascospores; d: Apical paraphyses; e: Immature (left) and mature asci. Scale bars: a: 100 µm; b: 25 µm; c–e: 15 µm.

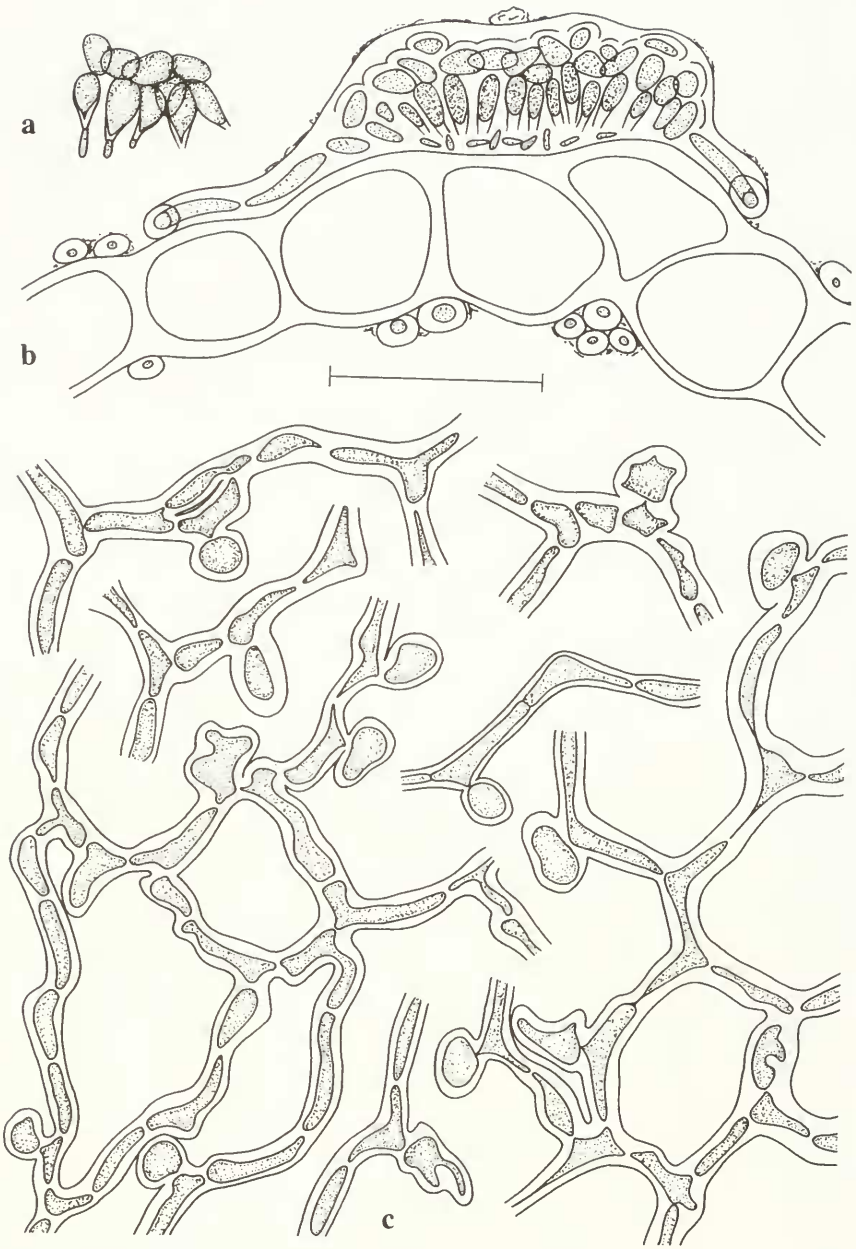

Fig. 2: *Bryonectria callicarpa* (M, holotype). a: Phialides; b: Section through dome-like structure of anamorphic state with phialides on leaf surface; c: Hyphae with appressoria. Scale bar: a–c: 20 µm.

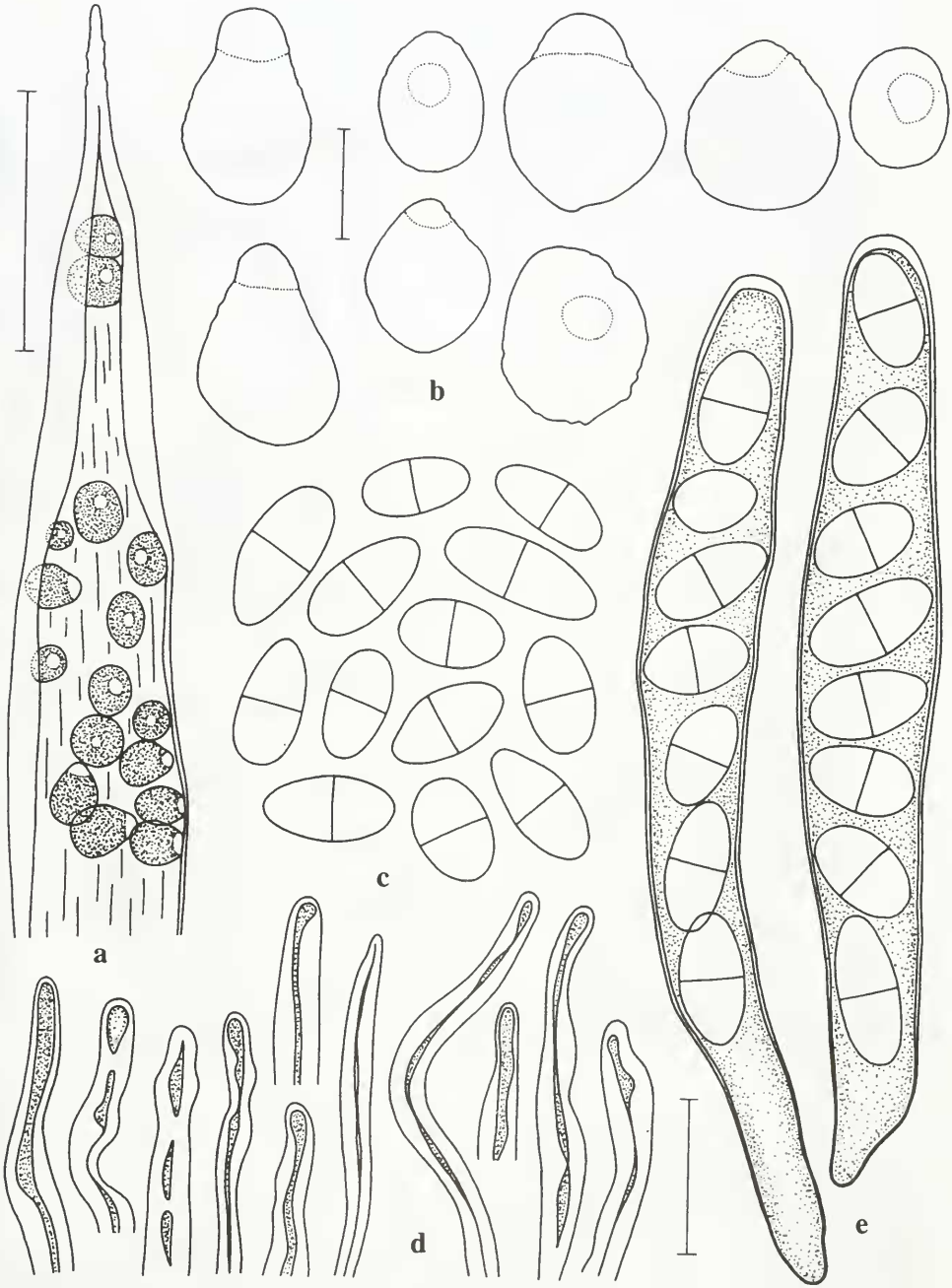

Fig. 3: *Bryonectria phyllogena* (M, holotype). a: Host leaf colonized by ascomata seen from above; b: Ascomata with paler apical region seen laterally or from above in outline; c: Ascospores; d: Hyphae covering the ascomatal wall; e: Asci. Scale bars: a: 1 mm; b: 150 µm; c–e: 15 µm.

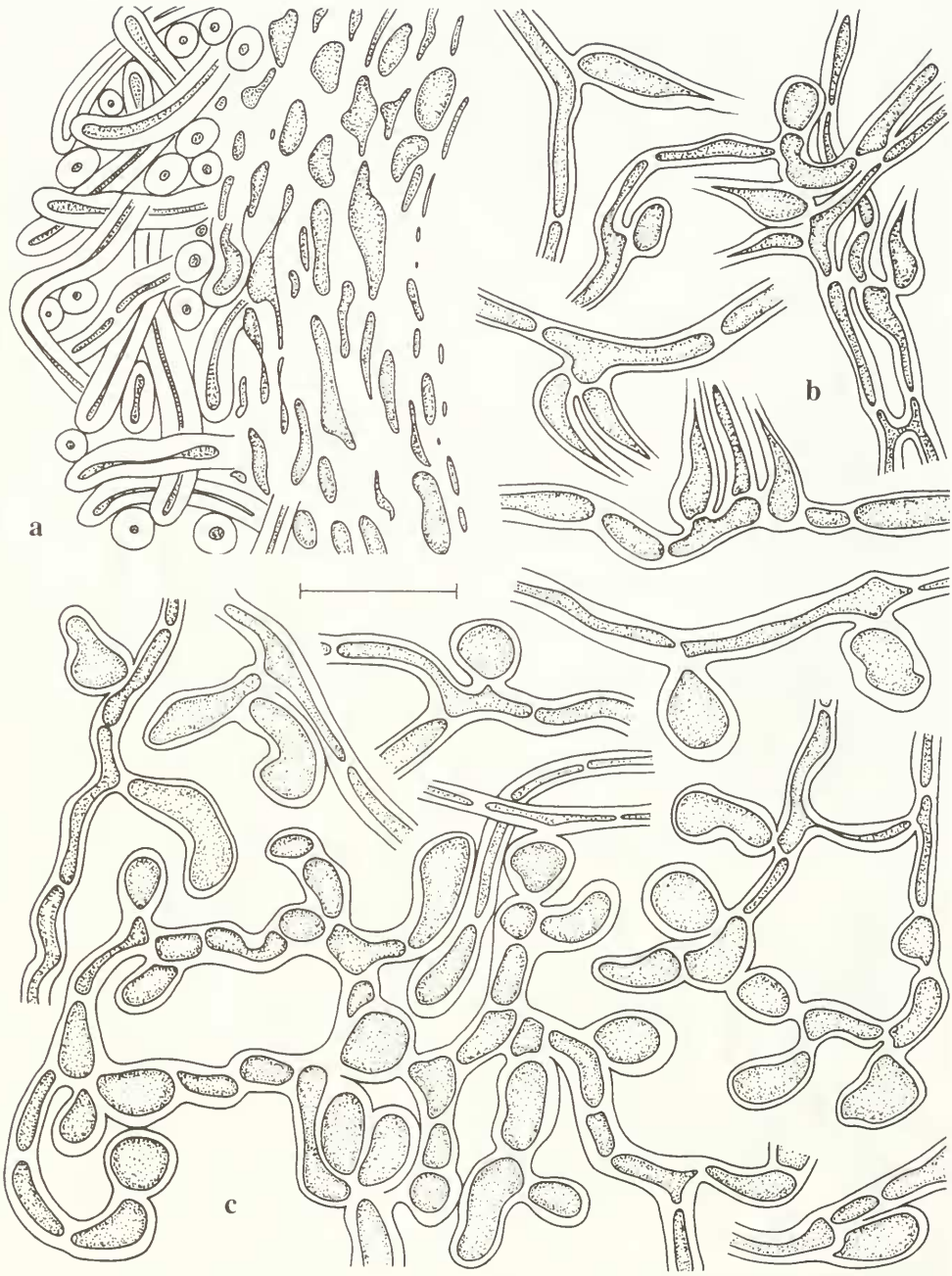

Fig. 4: *Bryonectria phyllogena* (M, holotype). a: Lateral ascomatal wall in longitudinal section; b: Hyphae with phialides; c: Hyphae with appressoria; Scale bar: a–c: 15 µm.